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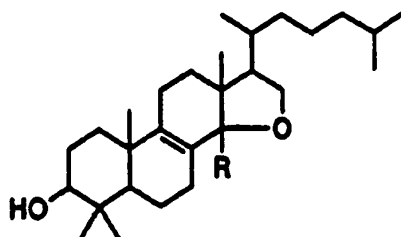
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<p>(21) International Application Number: PCT/US94/01191 (22) International Filing Date: 8 February 1994 (08.02.94) (30) Priority Data: 08/016,554 11 February 1993 (11.02.93) US (71) Applicant: THE DU PONT MERCK PHARMACEUTICAL COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). (72) Inventor: KO, Soo, Sung; 3215 Drexel Drive, Wilmington, DE 19810-3455 (US). (74) Agents: BOUDREAUX, Gerald, J. et al.; The Du Pont Merck Pharmaceutical Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).</p>		<p>(81) Designated States: AU, CA, JP, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.</p>

(54) Title: 15-OXASTEROLS AS HYPOCHOLESTEROLEMICS



(1)

(57) Abstract

This invention provides compounds, compositions thereof, and methods of using said compounds and compositions in treating hypercholesterolemia in a mammal. Compounds of this invention have been shown to inhibit the biosynthesis of cholesterol and have potential for decreasing cholesterol formation in mammals. Compounds of the present invention have formula (1), wherein R is  $\text{CH}_2\text{CH}_3$ ,  $\text{CHOHCH}_2\text{CH}_3$ , or  $\text{CH}_2\text{CH}=\text{CH}_2$ .

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TITLE

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15-OXASTEROLS AS HYPOCHOLESTEROLEMICS

Field of the Invention

10 This invention relates to novel 15-oxasterols, pharmaceutical compositions thereof, and methods of using said compositions in mammals to inhibit the biosynthesis of cholesterol.

15

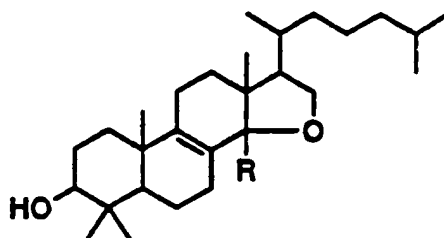
BACKGROUND OF THE INVENTION

Ko and Trzaskos, International Patent Application (PCT) WO 91/13903, published on September 19, 1991, disclose a class of 15-oxasterol cholesterol  
20 biosynthesis inhibitors useful as cholesterol lowering agents. The application of Ko and Trzaskos discloses 15-oxa, thia and azasterols substituted at the 14-position of the sterol nucleus with alkyl, alkenyl or alkynyl, which can themselves be substituted with  
25 oxygen, sulfur, nitrogen and other functional groups.

The general disclosure of WO 91/13903 encompasses the compounds of this invention, however, the compounds of this invention are neither exemplified nor  
30 specifically claimed in WO 91/13903. Applicants have now discovered that the compounds of the present invention exhibit remarkable and unexpected potency as cholesterol biosynthesis inhibitors, and are unexpectedly superior to compounds disclosed in WO 91/13903.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides 15-oxasterol compounds which exhibit remarkable and unexpected potency as cholesterol biosynthesis inhibitors. The compounds of this invention are 15-oxasterols of formula 1:



formula 1

wherein:

R is  $\text{CH}_2\text{CH}_3$ ,  $\text{CHOHCH}_2\text{CH}_3$ , and  $\text{CH}_2\text{CH}=\text{CH}_2$ .

This invention also provides pharmaceutical compositions comprising compounds of formula 1. Finally, this invention provides a method of treating hypercholesterolemia by orally administering a compound of formula 1.

As used herein, the term "therapeutically effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human subject that is being sought by a clinician or researcher.

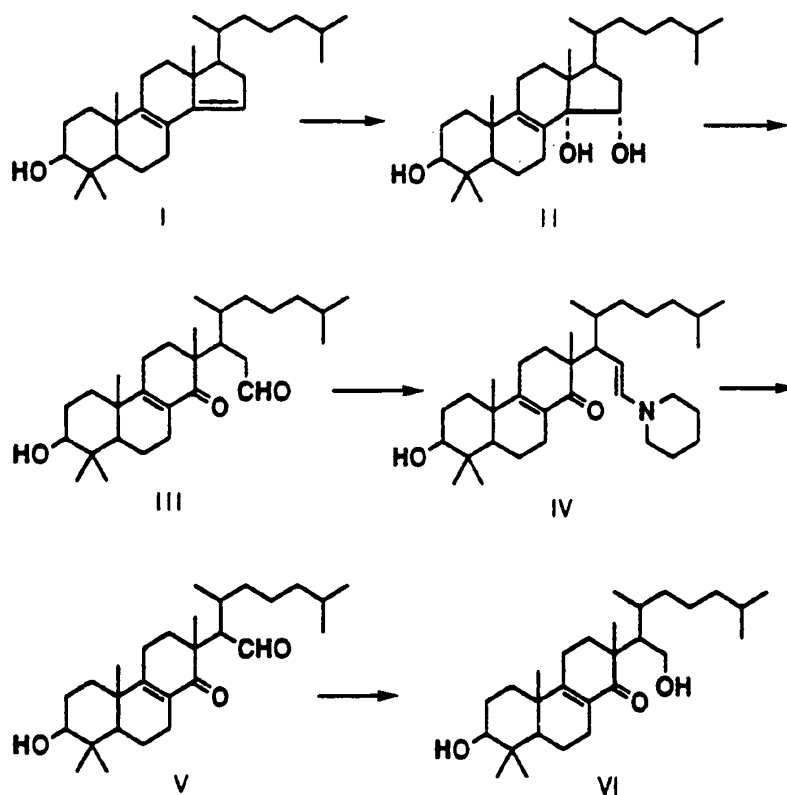
Synthesis

The compounds of formula 1 are prepared using methods disclosed in Schemes 1 and 2. As shown in Scheme 1, the starting material, 4,4-dimethyl-5a-cholesta-8,14-dien-3b-ol (I) [Bloch and Gautschi, *J. Chem. Soc.*, 223, (6), 1343 (1958)] was hydroxylated by a catalytic osmium tetroxide reaction in a mixture of dioxane and water using trimethylamine-N-oxide as an

oxidant in the presence of pyridine and diazabicyclo-  
[4,4,0]undec-7-ene (DBU) to give the triol (II). Next,  
the D-ring of the sterol was opened by treating the  
triol with either lead tetraacetate in benzene or sodium  
5 periodate in aqueous methanol to provide the  
corresponding enone-aldehyde (III). The aldehyde-  
carrying chain was shortened by converting the aldehyde  
to an enamine (IV) and reacting the enamine with ozone  
followed by dimethylsulfide in dichloromethane at -78 °C  
10 to give a new enone-aldehyde (V). After replacing the  
solvent with methanol the aldehyde was treated with  
sodium borohydride to provide the corresponding enone-  
alcohol (VI), which serves as the key intermediate for  
the 15-oxasterols of this invention.

15

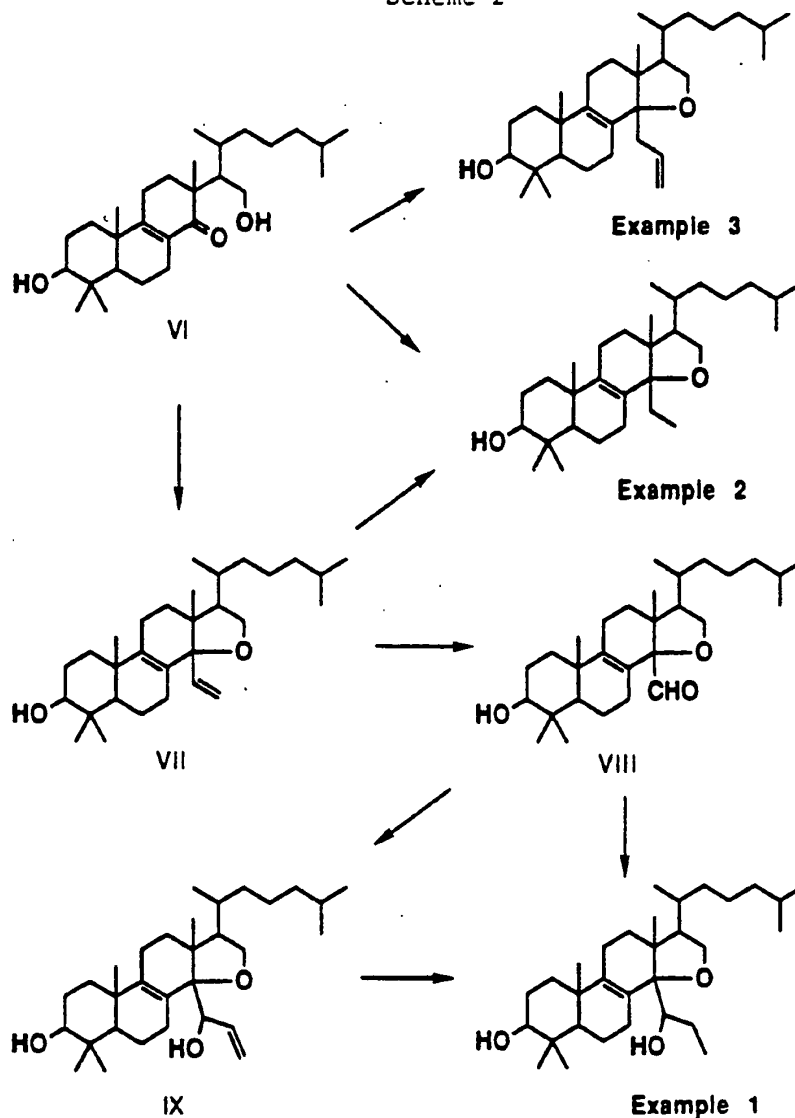
Scheme 1



20 As shown in Scheme 2, compounds of formula 1 are  
then prepared by nucleophilic addition to the enone-

alcohol (VI) using either a Grignard reagent or an alkyl lithium reagent in an inert solvent such as diethyl ether, tetrahydrofuran or in a mixture of inert solvents; and treating the product with an acid in an alcoholic solvent such as methanol or ethanol. The primary hydroxyl group of the enone-alcohol (VI) can be masked with a suitable protecting group such as a silyl ether prior to the nucleophilic addition when necessary. Specifically, the compounds of Examples 2 and 3 are prepared by this method. Thus, reaction of (VI) with either ethyl magnesium bromide or ethyllithium provides the compound of the formula 1 wherein R is ethyl (Example 2); and reaction of (VI) with either allyl magnesium bromide or allyllithium provides a compound of formula 1 wherein R is allyl (Example 3).

Scheme 2



As shown in Scheme 2, the R group of formula 1 can be further elaborated when R is  $\text{CH}=\text{CH}_2$  (compound VII) to prepare additional examples of this invention. For example, the vinyl group of (VII) can be hydrogenated to afford the corresponding ethyl compound which is the compound of the formula 1 wherein R is ethyl (Example 2), or it can be treated with ozone to yield the corresponding aldehyde (VIII). The aldehyde can then be reacted with either ethyl magnesium bromide or ethyllithium to afford the compound of formula 1 wherein

R is  $\text{CHOHCH}_2\text{CH}_3$  (Example 1). Alternatively, the compound of Example 1 compound can be prepared by reacting the aldehyde (VIII) with vinyl magnesium bromide or vinyl lithium to provide Compounds IXa and IXb, and then reducing the vinyl group by catalytic hydrogenation.

#### EXAMPLES

10 Syntheses of specific examples are shown below.

##### Synthesis of Intermediate Compound VI

15 Step 1: Preparation of 4,4-Dimethyl-5 $\alpha$ -cholest-8-ene-3 $\beta$ ,14 $\alpha$ ,15 $\alpha$ -triol (Compound II).

To a stirred solution of 4,4-dimethyl-5 $\alpha$ -cholesta-8,14-dien-3 $\beta$ -ol (Compound I, 254 g, 0.615 moles) in 1,4-dioxane (2500 ml) were added pyridine (245 ml), 1,8-diazabicyclo[4.4.0]undec-7-ene (DBU, 100 ml) an aqueous solution of trimethylamine N-oxide dihydrate (135 g, 1.21 moles in 500 ml of water) and a 20% solution of osmium tetroxide in dichloromethane (64 ml, 0.05 moles), and the mixture was stirred at 80-90 °C for 16 hours. After cooling to room temperature was added 20% solution of sodium bisulfite (1000 ml) and the mixture was stirred at room temperature for 2 hours. It was partitioned between ethyl acetate (2000 ml) and water (1000 ml) and the two layers were separated. The aqueous layer was extracted with ethyl acetate (3 x 1000 ml) and the combined extracts were washed with water and brine. The ethyl acetate solution was dried over magnesium sulfate and evaporated to give a solid residue. The crude product was dissolved in a minimum amount of ether and passed through a short column of silica gel (1000 g) with elution by ether to remove the colored impurities. The solvent was evaporated to



afford a white crystalline solid of the desired triol (Compound II) in about 80% yield.

M.P. 133-134 °C (decomposed), NMR [300 MHz, CDCl<sub>3</sub>-D<sub>2</sub>O (5%)]  $\delta$  4.11 (dd, J=5 Hz, 9 Hz, 1H), 3.22 (dd, J=5 Hz, 11 Hz, 1H), 2.33 (m, 1H), 2.26 (brm, 1H), 2.09 (brm, 2H), 2.00-1.05 (m, 20H), 1.01 (d, J=3 Hz, 6H), 0.87 (s, 3H), 8.86 (d, J=8 Hz, 3H), 0.85 (s, 3H), 0.82 (s, 3H), 0.69 (s, 3H); IR (film) : 3422 (bs), 2950 (s), 1652 (s), 1465 (m), 1036 (m) cm<sup>-1</sup>; EA for C<sub>29</sub>H<sub>50</sub>O<sub>3</sub> : Calcd. C 77.97%, H 11.2%; found C 78.05%, H 11.19%.

Step 2: Preparation of 4,4-Dimethyl-3 $\beta$ -hydroxy-15-oxo-5 $\alpha$ -secocholest-8-en-14-one (Compound III).

15

To a stirred solution of the triol (Compound II, 111.6 g, 0.25 moles) in dry benzene (3500 ml) in the dark was added lead tetracetate (113.1 g, 0.25 moles) in 7 roughly equal portions over a period of 1 hour and the mixture was continued to stir for an additional hour. The mixture was filtered through Celite and the filtercake was rinsed several times with benzene. Evaporation of the solvent provided a foamy solid of the enone-aldehyde (Compound III) in near quantitative yield.

25

NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  3.93 (t, J=8.1 Hz, 1H), 3.63 (t, J=8.1 Hz, 1H), 3.26 (dd, J=4.8 Hz, 11.4 Hz, 1H), 2.10-0.70 (m, 25H), 1.01 (s, 3H), 0.98 (s, 3H), 0.95 (d, J=6.3 Hz, 3H), 0.89 (s, 3H), 0.88 (d, J=6.6 Hz, 6H), 0.81 (s, 3H); IR (film) : 3336, 2932, 2870, 1467, 1458, 1383, 1377, 1266, 1040, 1028, 924, 737 cm<sup>-1</sup>.

30

Step 3: Preparation of 4,4-Dimethyl-3 $\beta$ ,16-dihydroxy-14,16-seco-D-nor-cholest-8-en-14-one (Compound VI).

35

To a solution of the enone-aldehyde (Compound III, 111.2 g, 0.25 moles) in benzene (1500 ml) was added

piperidine (66.8 ml, 0.68 moles) and the mixture was refluxed under a Dean-Stark trap until the removal of water is completed (1-1.5 hours). The excess piperidine and the solvent were evaporated off under reduced  
5 pressure after cooling to give a foamy solid residue of the enamine (Compound IV).

The enamine was dissolved in dichloromethane (1750 ml) and the solution was cooled to -78 °C. Ozone was  
10 bubbled through the solution and reaction was followed by thin layer chromatography (TLC) using silica gel plate and a 7:93 mixture of acetone and toluene. Near the end point of the reaction was added few drops of Sudan Red 7B in dichloromethane and ozone was continued  
15 to bubble through until the color become light pink. After stirring for 5 minutes, was added dimethyl sulfide (60 ml) and the mixture was stirred at -78 °C for 30 minutes. The residue after evaporation of the solvent was dissolved in a minimum amount of ether and passed  
20 through a plug of silica gel with elution by a 1:9 mixture of ethyl acetate and hexane to remove polar impurities. Evaporation of the solvent afforded a foamy solid of the enone-aldehyde (Compound V, 98 g).

25 The enone-aldehyde (Compound V) was dissolved in methanol (995 ml) and the solution was cooled to -40 °C. Then was added sodium borohydride (5.16 g, 0.136 moles) in a single portion and the mixture was stirred for 1.5 hours while allowing the temperature to rise to -10 °C.  
30 The excess sodium borohydride was destroyed by dropwise addition of saturated aqueous ammonium chloride solution over a period of 15 minutes. The solution was then made basic with saturated solution of sodium bicarbonate and concentrated under reduced pressure. The residue was  
35 extracted with ethyl acetate (3 x 500 ml) and the combined extracts were washed with water and brine, dried over magnesium sulfate, and evaporated to give an oily residue of the enone-alcohol (Compound VI) in about 90% yield as a crude product.

NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  3.72 (m, 1H), 3.56 (m, 1H), 3.28 (m, 1H), 2.60 (dd,  $J=6.0$  Hz, 18.1 Hz, 1H), 2.37-0.8 (m, 23H), 1.07 (s, 6H), 1.06 (d,  $J=5.2$  Hz, 3H), 1.02 (s, 3H), 0.86 (d,  $J=6.0$  Hz, 6H), 0.84 (s, 3H); IR (film) : 3426, 2951, 2869, 1651, 1625, 1462, 1374, 1094, 1029, 733  $\text{cm}^{-1}$ .

#### Example 1

10

#### 32-Ethyl-15-oxa-lanost-8-ene-3 $\beta$ ,32-diol (Compound 1)

Step 1: Preparation of 4,4-Dimethyl-15-oxa-14-vinyl-5 $\alpha$ -cholest-8-en-3 $\beta$ -ol (Compound VII).

15

To a stirred solution of the enone-alcohol (Compound VI, 140 g, 0.324 moles) in dry tetrahydrofuran (1670 ml) were added 2,6-lutidine (79.3 ml, 0.68 moles) and 4-dimethyl-aminopyridine (1.56 g), and the mixture was cooled to 0 °C. Then chlorotrimethylsilane (82.4 ml, 0.65 moles) was added dropwise over a period of 10 minutes and it was stirred at the same temperature for 1 hour. To the mixture was added dropwise 1M-vinyl magnesium bromide in tetrahydrofuran (1621 ml, 1.621 moles) and it was gently refluxed for 1 hour. After cooling to 0 °C, 2N-HCl was added dropwise with caution and the mixture was stirred for 30 minutes. Two layers were separated and the aqueous layer was extracted with ethyl acetate (2 x 500 ml). The combined organic extracts were washed with brine, dried over magnesium sulfate and evaporated to give an oily residue. The residue was then dissolved in methanol (1624 ml) and camphorsulfonic acid (31g) was added. The mixture was stirred at room temperature for 4.5 hours and was made basic with saturated sodium bicarbonate solution. It was extracted with ethyl acetate (4 x 500 ml) and the combined extracts were washed with brine, dried over magnesium sulfate and evaporated to give an oily residue. The crude product was purified by column

chromatography on silica gel with elution by a 5 : 95 mixture of ethyl acetate and hexane to provide 86 g of pure vinyl-oxasterol (Compound VII).

5 NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.73 (dd,  $J=10.8$  Hz, 17.4 Hz, 1H), 5.12 (dd,  $J=1.8$  Hz, 10.8 Hz, 1H), 5.00 (dd,  $J=1.8$  Hz, 17.4 Hz, 1H), 3.88 (t,  $J=8.7$  Hz, 1H), 3.55 (t,  $J=9$  Hz, 1H), 3.26 (dd,  $J=4.8$  Hz, 11.7 Hz, 1H), 2.07-0.80 (m, 23H), 1.01 (s, 6H), 0.95 (d,  $J=6$  Hz, 3H), 0.86 (d,  $J=6.3$  Hz, 6H), 0.81 (s, 3H), 0.78 (s, 3H); IR (film) : 2933, 2868, 1512, 1467, 1375, 1095, 1040, 1033, 1029  $\text{cm}^{-1}$ ; HRMS for  $\text{C}_{30}\text{H}_{50}\text{O}_2$  ( $\text{M}^+$ ) : Calcd. 442.3811; found 442.3806.

15 Step 2: Preparation of 3 $\beta$ -Hydroxy-15-oxa-lanost-8-en-32-al (Compound VIII).

A solution of the vinyl-oxasterol (Compound VII, 1.96 g, 4.4 mmoles) in methanol (80 ml) at  $-78^\circ\text{C}$  was bubbled with ozone until the solution turn to light blue. After stirring for 3 minutes the excess ozone was purged out and the mixture was treated with dimethylsulfide (1 ml). The temperature was raised to  $0^\circ\text{C}$  and the solvent was evaporated off to give an oily residue. It was purified by a column chromatography 20 with elution by a 1:9 mixture of ethyl acetate and hexane to give 1.15 g of pure aldehyde (Compound VIII).

30 NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  9.59 (s, 1H), 4.03 (t,  $J=8.4$  Hz, 1H), 3.70 (dd,  $J=8.1$  Hz, 9.6 Hz, 1H), 3.26 (dd,  $J=4.5$  Hz, 11.4 Hz, 1H), 2.20-0.90 (m, 24H), 1.04 (s, 3H), 1.01 (s, 3H), 0.95 (d,  $J=6.6$  Hz, 3H), 0.92 (s, 3H), 0.86 (d,  $J=6.3$  Hz, 6H), 0.81 (s, 3H); IR (film) : 3446, 2946, 2933, 2870, 2691, 1731, 1468, 1383, 1378, 1365, 1064, 1042, 1027  $\text{cm}^{-1}$ ; HRMS for  $\text{C}_{28}\text{H}_{47}\text{O}_2$  ( $\text{M}-\text{CHO}$ ) : Calcd. 415.3576; found 415.3600.

Step 3: Preparation of 15-Oxa-32-vinyl-lanost-8-ene-3 $\beta$ ,32-diol (Compounds IXa and IXb).

To a solution of the aldehyde (Compound VIII, 14.37 g, 0.032 moles) in dry tetrahydrofuran (650 ml) at -78 °C was added 1M vinyl magnesium bromide in tetrahydrofuran (97 ml, 0.097 moles) dropwise over a period of 1.75 hours and the mixture was stirred for 2.5 hours while raising the temperature to 4 °C gradually. Then the reaction was quenched with saturated ammonium chloride. After addition of 1N-HCl (100 ml) the product was extracted with ethyl acetate (3 x 300 ml) and the combined extracts were washed with saturated sodium bicarbonate and brine. It was dried over magnesium sulfate and evaporated to give an oily residue (13.3 g) of 1:2 mixture of two epimeric allylic alcohols (Compounds IXa and IXb). The two epimers were separated by a column chromatography on silica gel with elution by a 7:93 mixture of ethyl ether and toluene.

Fast epimer (Compound IXa)

NMR (300 MHz, CDCl<sub>3</sub>) : δ 5.95 (m, 1H), 5.29 (dt, J=17,2 Hz, 1.8 Hz, 1H), 5.12 (dt, J=11.1 Hz, 2.2 Hz, 1H), 4.51 (m, 1H), 3.86 (t, J=8.4 Hz, 1H), 3.56 (dd, J=9.6 Hz, 8.4 Hz, 1H), 3.26 (m, 1H), 2.50 (d, J=2.9 Hz, 1H), 2.35-0.8 (m, 23H), 1.03 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 0.93 (d, J=6.6 Hz, 3H), 0.86 (d, J=6.6 Hz, 6H), 0.82 (s, 3H); IR (film) : 3459, 3090, 2939, 2869, 1642, 1466, 1416, 1375, 1042 cm<sup>-1</sup>; MS (CI) : 473 (M+H), 471 (M-H), 455 (M+H-H<sub>2</sub>O).

Slow epimer (Compound IXb)

NMR (300 MHz, CDCl<sub>3</sub>) : δ 5.70 (m, 1H), 5.27 (d, J=17.9 Hz, 1H), 5.12 (d, J=10.2 Hz, 1H), 4.32 (d, J=6.9 Hz, 1H), 3.87 (t, J=8.4 Hz, 1H), 3.51 (t, J=9.7 Hz, 1H), 3.25 (dd, J=6.4 Hz, 4.8 Hz, 1H), 2.56 (m, 1H), 2.13 (m, 23H), 1.01 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.94 (d, J=6.6 Hz, 3H), 0.86 (d, J=6.6 Hz, 6H), 0.81 (s, 3H); IR (film) : 3450, 3078, 2935, 2869, 1642, 1468, 1377, 1284, 1251, 1096, 1039 cm<sup>-1</sup>;

MS (CI) : 473 (M+H), 471 (M-H), 455 (M+H-H<sub>2</sub>O).

Step 4: Preparation of 32-Ethyl-15-oxa-lanost-8-ene-3 $\beta$ ,32-diol (Example 1).

5

A solution of the allylic alcohol (slow isomer, Compound IXb, 0.13 g) and 10% palladium on carbon (c.a. 50 mg) in ethyl acetate (10 ml) and acetic acid (0.5 ml) was stirred under hydrogen (1 atm.) at room temperature for 6 hours. After the stirring the mixture was filtered through Celite™ and the filtrate was evaporated to give an oily residue of the propyl alcohol (Compound 1). It was purified by a column chromatography on silica gel with elution by a 1:9 mixture of ethyl acetate and hexane.

10  
20  
NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  3.85 (t, J=8.4 Hz, 1H), 3.77 (bd, J=10.3 Hz, 1H), 3.48 (t, J=8.8 Hz, 1H), 3.25 (qn, J=5.9 Hz, 1H), 2.91 (s, 1H), 2.60 (dd, J=15.7 Hz, 6.2 Hz, 1H), 2.15-0.8 (m, 24H), 1.00 (d, J=5.1 Hz, 6H), 0.99 (t, J=6.9 Hz, 3H), 0.95 (d, J=6.6 Hz, 3H), 0.93 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.81 (s, 3H).

#### Example 2

25

32-Methyl-15-oxa-lanost-8-en-3 $\beta$ -ol (Compound 2)

A solution of the vinyl-oxasterol (Compound VII, 0.2 g) and 10% palladium on carbon (about 50 mg) in a 20:1 mixture of ethyl acetate and acetic acid (10 ml) was stirred under hydrogen (1 atm.) at room temperature for 16 hours. At the end of the stirring the mixture was filtered through Celite® and the filtrate was evaporated to give an oily residue of the ethyl-oxasterol (Compound 2). It was purified by a column chromatography on silica gel with elution by a 1:9 mixture of ethyl acetate and hexane.

NMR (300 MHz, CDCl<sub>3</sub>) :  $\delta$  3.76 (t, J=8.78 Hz, 1H), 3.42 (t, J=8.61 Hz, 1H), 3.27 (m, 1H), 2.11-0.8 (m, 25H), 1.01 (d, J=5.13 Hz, 6H), 0.95 (d, J=7.7 Hz, 3 H), 0.94 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H), 0.74 (t, J=8.01 Hz, 3H).

### Example 3

#### 15-Oxa-32-vinyl-lanost-8-en-3 $\beta$ -ol (Compound 3)

10

To a stirred solution of the enone-alcohol (Compound VI, 2 g, 4.62 mmoles) in dry tetrahydrofuran (30 ml) were added 2,6-lutidine (1.13 ml, 9.7 mmoles) and 4-dimethyl-aminopyridine (22 mg), and the mixture was cooled to 0 °C. Then chlorotrimethylsilane (1.18 ml, 9.3 mmoles) was added dropwise over a period of 10 minutes and the mixture was stirred at the same temperature for 30 minutes. To the mixture was added dropwise 1.2M allyl magnesium bromide in tetrahydrofuran (19.3 ml, 23.1 mmoles) and it was stirred at room temperature for 3 hours. The reaction was quenched with 1N HCl (30 ml) and it was extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with brine, dried over magnesium sulfate and evaporated to give an oily residue. The residue was then dissolved in methanol (25 ml) and camphorsulfonic acid (0.45 g) was added. The mixture was stirred at room temperature for 4 hours and was made basic with saturated sodium bicarbonate solution. It was extracted with ethyl acetate (3 x 50 ml) and the combined extracts were washed with brine, dried over magnesium sulfate and evaporated to give an oily residue. The crude product was purified by column chromatography on silica gel with elution by a 5 : 95 mixture of ethyl acetate and hexane to provide the pure allyl-oxasterol (Compound 3).

NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.57 (m, 1H), 5.01 (d, J=17.2 Hz, 1H), 4.94 (d, J=11.7 Hz, 1H), 3.77 (t, J=8.61 Hz, 1H),

3.43 (t, J=8.60 Hz, 1H), 3.26 (q, J=5.90 Hz, 1H), 2.58  
(dd, J=14.6 Hz, 4.9 Hz, 1H), 2.36 (dd, J=14.6 Hz, 9.9  
Hz, 1H), 2.16-0.8 (m, 23H), 1.01 (d, J=5.9 Hz, 6H), 0.94  
(s, 3H), 0.94 (d, J=5.1 Hz, 3H), 0.87 (s, 3H), 0.85 (s,  
5 3H), 0.82 (s, 3H).

### Utility

The cholesterol biosynthesis inhibition activities  
10 of the compounds of formula 1 were assayed by measuring  
<sup>14</sup>C-acetate incorporation into the biosynthesis of  
cholesterol as described in detail below. This assay is  
identical to that disclosed in Example 31 of WO 91/13903  
which is incorporated herein by reference. In addition,  
15 the 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase  
(HMGCR) Suppression Assay, disclosed in Example 26 of WO  
91/13903, and method "Lowering Blood Cholesterol Levels  
in Hamsters" of Example 27 of WO 91/13903 are also both  
incorporated herein by reference.

20

### Assay of <sup>14</sup>C-Acetate Incorporation into the Biosynthesis of Cholesterol

The HepG2 cells (a human hepatoma cell line) used  
25 in the assay were obtained from the American Type  
Culture Collection (ATCC, Rockville, MD). They were  
maintained in Dulbecco's Modified Eagles Medium and  
Ham's F12 Medium (1:1) supplemented with 10% heat  
inactivated Fetal Bovine serum, 10 mM Herpes, 1 mM  
30 sodium pyruvate, 1 x Non-essential Amino Acids (Gibco)  
and 2 mM L-glutamine.

The cells were harvested by washing cultures with  
10 ml Hank's Balanced Salt Solution (2x) and incubating  
with 0.125% Trypsin in Versene (0.02% EDTA) for  
35 approximately one minute. After the cells are visibly  
rounded and loosened from the flask, 9 ml of the above  
medium is added. The cells are transferred to a 100 mM  
culture dish and syringed up and down through a 20 gauge  
needle to break up aggregated clumps of cells. Cell



cultures are plated at  $0.1 \times 10^6/\text{ml}$  and  $0.075 \times 10^6/\text{ml}$  in two 24 well plates, respectively, aliquoting 1 ml cells/well. The cells are allowed to attach for 24 hours (48 hours for the lower density plate) before  
5 washing 2x with HBSS and refed with 1 ml of the above medium with 1% Cabosil delipidated serum instead of Fetal Bovine Serum. The cells are treated with the test compounds after 24 hours of exposure to the delipidated serum media.

10 Chemicals are routinely prepared as a 10 mM solution in 100% ethanol or dimethyl sulfoxide (DMSO). All test compounds are added at 50 mM, 25 mM, 10 mM, 1 mM at a final concentration of 0.45% solvent, 0.25% Bovine Serum Albumin (BSA) suspension per well. The solvent BSA is  
15 sonicated for 10 seconds to ensure maximum solubilization before addition to the cells. Control wells receive solvent/BSA at the same concentrations as the drug treated cultures. After incubation for one hour at 37 °C, 5%  $\text{CO}_2$ , 20 mCi/ml  $^3\text{H}$ -MVAL is added per  
20 well in ethanol/medium so the final concentration of solvent is 1.2%. After 22 hours of incubation with the test compounds 2.5 mCi of  $^{14}\text{C}$ -acetate is added per well for an additional 2 hours so that the final concentration of ethanol is 1.6%.

25 Two known cholesterol biosynthesis inhibitors are incubated with each assay, namely 25-hydroxycholesterol and Lovastatin, to determine the reliability and validity of each assay.

The cultures are harvested by aspirating the media  
30 and washing twice with ice cold 0.5 M Tris, 0.15 M NaCl, PH 7.4 to remove excess radiolabel not incorporated into the cells. Stop Reagent (1 ml of 15% potassium hydroxide, 85% methanol, 100 mg/ml butylated hydroxytoluene (BHT) is added to each well and the plate  
35 is sonicated in a mild water sonicating bath to release the cells from the bottom of the well. The digested cell extracts are transferred to 15 ml extraction tubes. Each well is rinsed with an additional 1 ml of the Tris/NaCl buffer which is added to the appropriate

xtraction tube. An aliquot (100 ml) is removed for protein determination at this point if desired.

The cell extracts are saponified at 80 °C for 30 minutes. After cooling 8 ml petroleum ether is added  
5 and the tubes are twirled on a rotary extractor for 5-10 minutes to extract the sterols into the organic solvent phase. The top organic phase is removed and passed through a Silica Seppak (Waters) which binds all sterols and free fatty acids. Sterols are eluted with a 5 ml  
10 diethyl ether:hexane (1:1) rinse. This sterols extraction is automated using Millilab (Waters) to ensure reproducibility and accuracy from sample to sample.

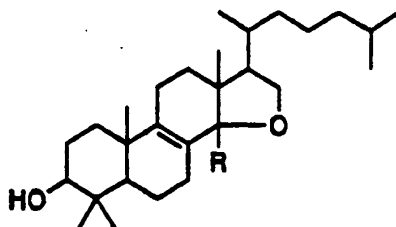
The eluted sterols are dried under nitrogen gas and  
15 resuspended in 150 ml ethanol. A 15 ml aliquot is removed from each sample and added to a scintillation vial filled with formula 989 (NEN). The samples are counted on the dual label  $^3\text{H}$ : $^{14}\text{C}$  program on the Beckman scintillation counter Model LS 7800.

20 The incorporation of both radiolabeled precursors into sterols is compared between the treated and non-treated (control) cultures and expressed as "% control" for each precursor. Test compounds are classified as being "active" or "inactive" from these results and  $\text{IC}_{50}$   
25 values were determined for the active compounds.

Analysis of sterol profiles is performed on the remainder of the sample by reversed phase HPLC. Analyses are done using an Ultrasphere octyl column (Altex) (0.46 x 25 cm), with a mobile phase consisting  
30 of acetonitrile:methanol: $\text{H}_2\text{O}$  (44.5:44.5:10). Chromatography is performed at a flow rate of 1.5 ml per minute at 45 °C.

Cholesterol biosynthesis inhibition activities of the compounds of formula 1, which were determined by the  
35 method described above, are shown in the Table 1. The  $\text{IC}_{50}$  values represent the level of inhibition of the incorporation of  $^{14}\text{C}$ -acetate into the cholesterol biosynthesis pathway.

Table 1



Example Number	R	Melting Point	IC <sub>50</sub> (mM)
1	CHOHCH <sub>2</sub> CH <sub>3</sub>	amorphous	0.001
2	CH <sub>2</sub> CH <sub>3</sub>	amorphous	0.003
3	CH <sub>2</sub> CH=CH <sub>2</sub>	amorphous	<0.05

5

The compounds of this invention have greater potency in cholesterol biosynthesis inhibition than any of the compounds specifically disclosed and tested in WO 91/13903. The compounds of this invention exhibited cholesterol biosynthesis inhibition activity approximately 2 to 80 fold higher than the most active compounds specifically disclosed in WO 91/13903 which have been tested. Thus, Applicant has shown the compounds of the present invention to be unexpectedly superior to those disclosed and tested in PCT application WO 91/13903 in an identicle assay. These results indicate that compounds of the present invention have excellent potential as effective hypocholesterolemic agents.

20

## Formulations

Compounds of this invention can be administered to treat said deficiencies by means that produces contact of the active agent with the agent's site of action in the body of a mammal. The compounds can be administered by any conventional means available for use in conjunction with pharmaceuticals either as individual therapeutic agent or in combination of therapeutic agents. They can be administered alone, but are generally administered with a

pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

5       The dosage administered will vary depending on the use  
and known factors such as pharmacodynamic character of the  
particular agent, and its mode and route of administration;  
the recipient's age, weight, and health; nature and extent  
of symptoms; kind of concurrent treatment; frequency of  
treatment; and desired effect. For use in the treatment of  
10   said diseases or conditions, the compounds of this  
invention can be orally administered daily at a dosage of  
the active ingredient of 0.002 to 200 mg/kg of body weight.  
Ordinarily, a dose of 0.01 to 10 mg/kg in divided doses one  
to four times a day, or in sustained release formulation  
15   was effective in obtaining the desired pharmacological  
effect.

Dosage forms (compositions) suitable for  
administration contain from about .1 mg to about 1000 mg of  
active ingredient per unit. In these pharmaceutical  
20   compositions, the active ingredient will ordinarily be  
present in an amount of about 0.5 to 95% by weight based on  
the total weight of the composition.

The active ingredient can be administered orally in  
solid dosage forms, such as capsules, tablets and powders;  
25   or in liquid forms such as elixirs, syrups,  
and/or suspensions. The compounds of this invention can  
also be administered parenterally in sterile liquid dose  
formulations.

Gelatin capsules can be used to contain the active  
30   ingredient and a suitable carrier such as but not limited  
to lactose, starch, magnesium stearate, steric acid, or  
cellulose derivatives. Similar diluents can be used to make  
compressed tablets. Both tablets and capsules can be  
manufactured as sustained release products to provide for  
35   continuous release of medication over a period of time.  
Compressed tablets can be sugar-coated or film-coated to  
mask any unpleasant taste, or used to protect the active  
ingredients from the atmosphere, or to allow selective  
disintegration of the tablet in the gastrointestinal tract.

Liquid dose forms for oral administration can contain coloring of flavoring agents to increase patient acceptance.

In general, water, pharmaceutically acceptable oils, saline, aqueous dextrose (glucose), and related sugar solutions and glycols, such as propylene glycol or polyethylene glycol, are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents, such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or in combination, are suitable stabilizing agents. Also used are citric acid and its salts, and EDTA. In addition, parenteral solutions can contain preservatives such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences", A. Osol, a standard reference in the field.

Useful pharmaceutical dosage-forms for administration of the compounds of this invention can be illustrated as follows:

#### Capsules

A large number of units capsules are prepared by filling standard two-piece hard gelatin capsules each with 100 mg of powdered active ingredient, 150 mg lactose, 50 mg cellulose, and 6 mg magnesium stearate.

#### Soft Gelatin Capsules

A mixture of active ingredient in a digestible oil such as soybean, cottonseed oil, or olive oil is prepared and injected by means of a positive displacement was pumped into gelatin to form soft gelatin capsules containing 100 mg of the active ingredient. The capsules were washed and dried.

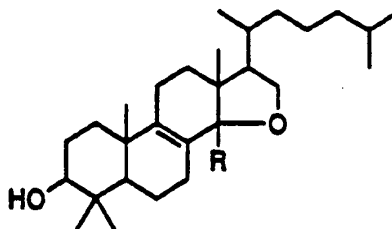
Tablets

A large number of tablets are prepared by conventional procedures so that the dosage unit was 100 mg active ingredient, 0.2 mg of colloidal silicon dioxide, 5 mg of  
5 magnesium stearate, 275 mg of microcrystalline cellulose, 11 mg of starch, and 98.8 mg lactose. Appropriate coatings may be applied to increase palatability or delayed adsorption.

The compounds of this invention may also be used as  
10 reagents or standards in the biochemical study of neurological function, dysfunction, and disease.

## WHAT IS CLAIMED IS:

1. A compound of formula:



5

wherein:

R is CH<sub>2</sub>CH<sub>3</sub>, CHOHCH<sub>2</sub>CH<sub>3</sub>, or CH<sub>2</sub>CH=CH<sub>2</sub>.

2. A pharmaceutical composition for decreasing  
10 cholesterol formation in mammals, said composition  
comprising a pharmaceutically acceptable carrier  
and a therapeutically effective amount of a  
compound of Claim 1.
- 15 3. A method of decreasing cholesterol formation in  
mammals in need of such therapy, said method  
comprising administering a therapeutically  
effective amount of a compound of Claim 1.

20

## INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/US 94/01191A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 C07J73/00 A61K31/58

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 13093 (THE DU PONT MERCK PHARMACEUTICAL COMPANY) 19 September 1991 see page 22 - page 49; example 31; table 1 see page 230; table I see page 246 -----	1-3

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

Date of the actual completion of the international search

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**Information on patent family members**

PCT/US 94/01191

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9113093	05-09-91	AU-B- 645077	06-01-94
		AU-A- 7496491	18-09-91
		EP-A- 0517826	16-12-92
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